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# Conductive adsorbents enhance phenol removal from wastewater by direct interspecies electron transfer "DIET"-based anaerobic biodegradation process

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ABSTRACT

This study examined the hybrid adsorption and anaerobic biodegradation processes of phenol by using activated carbon cloth (CC) to stimulate direct interspecies electron transfer (DIET). Phenol biodegradation was tested over three sequential runs using 100 mL lab-scale bioreactors and compared with two different types of CC and granular activated carbons (GAC). The addition of the carbon adsorbents to the bioreactors significantly accelerated the methane production rate. This high rate of methane production was also accompanied by the complete removal of phenol, and a high COD(s) removal rate of about 95%, compared to 43.68% and 48.65% in the control sets in the first and second runs, respectively. The enhancement of the phenol biodegradation is attributed to the synergy of the adsorption and biodegradation processes. An initial adsorption stage has contributed to a significant and rapid reduction of phenol, leading to a lower inhibition effect of phenol, followed by anaerobic biodegradation of the adsorbed phenol stimulated by the DIET process. Specifically, the optimal concentration for Single Weave carbon cloth (CCSW) of 1.6 g $L^{-1}$ promoted both the DIET and the adsorption processes. Microbial community composition analysis revealed that CCSW facilitated the growth of syntrophic bacterial groups (*Rikenellae*, *Syntrophorhabdaceae*, *Gracilibacteraceae*, and DTU014), and archaea (*Methanosaetaceae* and *Methanoregulaceae*), previously reported as key players in the DIET processes to promote phenol degradation. To the best of our knowledge, this is the first work that demonstrates the contribution of CC to stimulate DIET mechanism of anaerobic biodegradation synergistically combined with adsorption process for enhanced phenol removal.

## 1. Introduction

Phenol is a toxic intermediate chemical that is a common by-product of many industrial applications [1,2]. Phenol and its derivatives are widely found in the wastewater of many industries, i.e., pharmaceutical, coal conversion, chemical, and petrochemical industries, as well as oil refineries [3–5]. Wastewater with low to moderate concentrations of phenol (higher than 100 mg $L^{-1}$) [6–9] is considered a recalcitrant bio-toxin for microbes, inhibiting the biological processes in wastewater treatment plants (WWTPs) [2,3,10]. Much effort is being invested by industry and academia to effectively deal with the phenol problems [4]. Compared to other treatment technologies, anaerobic digestion (AD) has gained considerable attention for its numerous advantages, such as low energy consumption and low biomass yield, as well as its ability to promote energy recovery in the form of biogas [11,12]. Yet, two important factors: i) slow decomposition rate of volatile fatty acids (VFA) [13,14], and ii) low efficiency of indirect interspecies electron transfer (IIET) between syntrophic bacteria and methanogens [15,16], limit the conversion of phenol into $CH_4$ as the final biodegradation product. Therefore, it is expected that enhancing the rate of syntrophic metabolism will promote phenol biodegradation rates.

Direct interspecies electron transfer (DIET) is a process where free

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electron transfer between microbial cells, or between microbes and conductive materials. In contrast to IIET, DIET does not depend on reduced molecules such as hydrogen and formate [17]. Rather, it occurs through electrically conductive pili (e-pili), or direct contact using outer membrane c-type cytochromes [18,19] or through non-biological conductive materials such as activated carbons (AC) [20]. Thus, the three mechanisms: conductive e-pili, extracellular c-type cytochromes and conductive materials play a primary role in mediating DIET [14]. Since the synthesis of biological conductive structures by anaerobic microorganisms is energetically-expensive, utilizing AC as synthetic electron channels for DIET might be favorable for microbial growth [13,17]. It was recently discovered that the incorporation of carbon-based conductive materials such as graphite [17,21], biochar [1,21,22], granular activated carbon (GAC) [1,23], powdered activated carbon (PAC) [3], carbon fiber [24] and activated carbon cloth (CC) [15,20,23,25,26] in anaerobic bioreactors enhances syntrophic methanogenesis via DIET in addition to the adsorption processes. These conductive materials attribute to the acceleration, stabilization, and optimization of the AD process [1,3,13–15,17,20,23–28]. Their properties, such as superior chemical stability, good electrical conductivity, and high surface area can promote microbial growth, providing required nutrients to microbes, expediting interspecies electron transport, improving enzyme activity and buffering capacity, thus resulting in faster VFAs formation and utilization, faster $H_2$ and $CH_4$ production, shorter AD lag phase and higher $CH_4$ contents [29].

The large surface area and high porosity of these adsorbent materials can potentially uptake dissolved organic compounds like phenol to mitigate bio-toxicity in AD systems and promote the attachment of suspended microbes [1,30]. Compared with GAC or PAC, CC is reported as one of the most promising applicable additives in bioreactors [21,26] due to its high adsorption capacity and high conductive properties [20,31] as well as its physical architecture (membrane-like) that can be applied as a fixed easily replaceable unit [23,26,27,32]. The conductive property of CC was shown to enable the acceleration of the DIET mechanism and improve AD performance treating high concentrations of VFA wastewater [26]. Lei et al. [15] noted that the addition of CC increases the sludge conductivity from 5.47 to 9.77 $\mu Scm^{-1}$, possibly resulting from the enhancement of the DIET microorganisms. The CC was also used as an electrode to facilitate DIET [33].

While many studies have investigated the incorporation of conductive materials in anaerobic systems for simple substrates, such as ethanol [34], acetate [35], propionate [36], butyrate [37], and organic soil [25], the number of studies that tested the biodegradation of bio-toxic pollutants like phenol is limited, mainly using GAC and biochar, but not CC [1]. This study examines, for the first time, the impact of CC as a conductive adsorbent, stimulating DIET mechanism for the enhancement of the anaerobic biodegradation of phenol.

The objective of this study was to allow enhancement of phenol biodegradation stimulated by the DIET mechanism using CC that has a high electrical conductivity and an excellent adsorption efficiency.

To examine the effects of CC on enhancing the anaerobic biodegradation of phenol, biomethane potential (BMP) tests were performed with different AC configurations. Also, adsorption isotherms and kinetics experiments were conducted, zeta potential and sludge conductivity were measured, accompanied with microbial community analysis.

## 2. Materials and methods

### 2.1. Activated carbons

Two types of carbon cloth (CC) were used in this study, both having similar physicochemical properties, except for the electrical conductivity (EC) values: Carbon Cloth-Single Weave FM10 (CCSW) and Carbon Cloth-Knitted FM30K (CCK). For comparison, Granular Activated Carbon (GAC), Filtrasorb 300 GAC, was examined. This type of GAC was chosen because it is commonly used in the water treatment industries [38]. All three types of AC were purchased from Charcoal House in Colorado, USA, and their physicochemical properties are shown in Table 1.

### 2.2. Adsorption isotherms of phenol

Adsorption tests were conducted in 60 mL amber bottles. GAC, CCK, and CCSW were added in 1.6 g/L dosages into the amber bottles with different phenol concentrations of 0–450 mg/L. The phenol, purchased from Sigma-Aldrich, Israel, was diluted in distilled water (pH=7.3). Additional information about the phenol is shown in Table S1 (Supplementary Material). Each CC specimen was cut in a square shape which area was 6.25 $cm^2$ (2.5 cm×2.5 cm), while the GAC had an effective size of 0.8–1.0 mm. All bottles, with triplicates for each concentration, were incubated in an orbital shaker at 25 °C and 150 rpm for 18 hours. Then, 5 mL homorganic samples from each bottle were centrifuged at 10,000 rpm for 3 minutes, in order to sink the residual AC. The equilibrium concentration ($C_e$) of each sample was then calculated using a spectrophotometer (Thermo Scientific Evolution 260 Bio) at 270 nm and a calibration curve. The adsorption capacity ($q_e$) was calculated using Eq. 1.

$$q_e = \frac{V(C_i - C_e)}{M} \tag{1}$$

where $q_e$ is the adsorption capacity at equilibrium (mg/g), $C_i$ is the initial phenol concentration (mg/L), $C_e$ is the phenol concentration at equilibrium (mg/L), $V$ is the volume (L), and $M$ is the amount of AC (g). The constants of the Langmuir and Freundlich models [39,40] were calculated using Prism 8.4.3 for nonlinear regression, along with Eqs. 2 and 3, respectively:

$$q_e = \frac{q_m b C_e}{1 + bC_e} \tag{2}$$

$$q_e = K{C_e}^{1/n} \tag{3}$$

where $q_m$ is the maximum adsorption capacity (mg/g), $b$ is the Langmuir constant (L/mg), $K$ is the Freundlich constant, and $1/n$ is the heterogeneity factor.

### 2.3. Biomethane Potential (BMP) tests

#### 2.3.1. Optimization of CC concentration

To investigate the effects of the adsorption and conductive properties of CC on anaerobic phenol biodegradation, BMP batch tests were conducted [41]. Anaerobic granular sludge (inoculum) was taken from the up-flow anaerobic sludge blanket (UASB) bioreactor of the PRIGAT plant, in Kibbutz Givat Haim, Israel, which treats wastewater from the production of juices and syrups. The sludge was kept at 37 °C and washed 3 times in tap water before use, to avoid the presence of residual organic materials. Small batch systems consisting of 100 mL syringes, each connected to a three-way Luer lock adapter, were used [42]. The syringes (bioreactors) had an active volume of 60 mL medium and 6 g sludge (10% of the medium weight). The composition of the medium was: 1 g $L^{-1}$ $NH_4Cl$; 0.1 g $L^{-1}$ NaCl; 0.1 g $L^{-1}$ $MgCl_2$*6 $H_2O$; 0.05 g $L^{-1}$ $CaCl_2$*2 $H_2O$; 0.03 g $L^{-1}$ $K_2HPO_4$; 0.002 g $L^{-1}$ $FeCl_2$*4 $H_2O$;

**Table 1**
Physicochemical properties of the AC. Data provided by Charcoal House.

| | GAC | CCK | CCSW |
|---|---|---|---|
| Thickness [mm] | 0.5–2.5 | 0.4 | 0.5 |
| Electrical conductivity [$Sm^{-1}$] | — | 62.09 ± 3.07 | 230.5 ± 5.48 |
| Resistance [Ω] | — | 45.2 ± 2.24 | 12.2 ± 0.3 |
| Surface density [$gm^{-2}$] | — | 110 | 120 |
| Surface area [$m^2g^{-1}$] | 950 | 1200 | 1200 |
| Density in compression [$gcm^{-3}$] | 56 | — | — |

0.05 mg $L^{-1}$ $ZnCl_2$; 0.05 mg $L^{-1}$ $MnCl_2$*4 $H_2O$; 0.05 mg $L^{-1}$ $H_3BO_3$; 0.05 mg $L^{-1}$ $(NH_4)_6Mo_7O_{24}$*4 $H_2O$; 0.05 mg $L^{-1}$ $AlCl_3$; 0.05 mg $L^{-1}$ $CoCl_2$*6 $H_2O$; 0.9 mg $L^{-1}$ $NiCl_2$*6 $H_2O$; 0.5 mg $L^{-1}$ EDTA; 0.001 mg $L^{-1}$ HCl 37%; 0.1 mg $L^{-1}$ $NaSeO_3$*5 $H_2O$; 2600 mg $L^{-1}$ $NaHCO_3$; 250 mg $L^{-1}$ $Na_2S$; 100 mg $L^{-1}$ dry yeast; and 160 mg $L^{-1}$ peptone.In addition, 500 mg $L^{-1}$ of phenol and CCSW in a concentration range of 1–20 g $L^{-1}$ were added (corresponding to a surface of 3.75–75 $cm^{-2}$) to find the optimal CC concentration. 500 mg $L^{-1}$ was chosen to indicate a concentration which is higher than the inhibition level of phenol to microorganisms (higher than 100 mg $L^{-1}$) [6–9]. A syringe without CCSW (only medium, granular sludge, and phenol) was set up as the control treatment. The CCSW was cut into three pieces. Fig. 1 illustrates the BMP syringe that was used in this study. The reactors were flushed with 99.999% $N_2$ for 4 minutes using a glass dropping pipette to remove remnant oxygen/air from the headspace. Thereafter, the pipette was quickly removed, and the syringe was assembled. Next, the extra $N_2$ was evacuated by opening the Luer adapter, then quickly closing it. After that, the bioreactors were incubated simultaneously at 37 °C for 45 days, in the same incubation chamber, with the same inoculum. Three replicate tests were conducted for each concentration of CCSW. The biogas volume was measured every day, and the range of the initial and final pH values was 8.0–8.5 for all reactors. Methane ($CH_4$) content was measured at the end of the BMP test. A minimal 10 mL of biogas was needed for the Gas Chromatography (GC) analysis. The gas from the syringe bioreactor was withdrawn by opening the Luer adapter, through a 0.2 μ nylon membrane filter connected to a gas-tight syringe, fitted with a Luer lock push shut-off valve used in the GC analysis (see Analytical analysis in 2.5).

### 2.3.2. BMP test with different AC types

After determining the optimal concentration of CC, additional BMP batch tests were conducted using the three different types of AC (CCSW, CCK and GAC), in addition to a control group without AC (Medium). To examine the inhibitive effect of phenol on the anaerobic microorganisms, another group containing sludge, and a medium without phenol, was used (Medium No phenol). The test was performed as described above, except that the concentrations of the AC were obtained from the optimal CC concentration test. When no biogas production was apparent, a fresh medium with 500 mg $L^{-1}$ of phenol was added, signifying the beginning of a new run (in total three runs during 58 days). Furthermore, pH, EC, $CH_4$ content, soluble chemical oxygen demand ($COD_{(S)}$), and residual phenol concentration were analyzed for each bioreactor at the end of every run. $CH_4$ production rates were calculated according to Eq. 4. For this equation each time interval was selected by the first point of reaching plateau for each set of experiments.

$$CH_4 \quad production \quad rate = \frac{Biogas[mL] * CH_4[\%]}{time[days]} \tag{4}$$

### 2.4. DNA extraction, amplicon sequencing and microbial community analysis

The BMP test previously described (Section 2.2.2) was repeated for DNA sampling and microbial community analysis. Four sample types were collected during the BMP experiment: (i) biofilm attached to the CCSW (referred to as Attached); (ii) suspended sludge (SS) from CCSW added to the bioreactor (referred to as SS-CCSW); (iii) SS from the bioreactor without AC as a blank group (referred to as SS-B); and (iv) SS that was used as inoculum for the BMP test (referred to as SS-Time 0). For the Attached, SS-CCSW, and SS-B groups, triplicates were made for each batch, and for the SS-Time 0 group, triplicates were made as well, resulting in a total of 30 samples. All samples were suspended in 0.1 M PBS and stored at −20 °C until analysis. DNA was extracted from the 30 samples using the DNeasy® PowerSoil® Pro Kit (QIAGEN, Italy), according to the manufacturer's instructions. The V3–V4 hypervariable region of the 16 S gene was amplified with the 341 F/806 R primers containing CS1 and CS2 linkers, using PCR T-Professional Thermocycler (Biometra, Germany). PCR reaction products were loaded onto a 1.5% Agarose gel for DNA visualization. High-throughput sequencing was performed using the Illumina Miseq V3 sequencer platform (Genomics and Microbiome Core Facility, Rush University, Chicago). The two 250-bp paired-end sequences were merged to obtain a single read. Quality-filtered reads were imported into QIIME 2 platform (v. 2020.2) [43], denoised, dereplicated, clustered and trimmed using the DADA2 plugin [44]. Taxonomic assignment of the ASVs was achieved with the q2_feature_classifier [45], against the Silva database (release 138–99) for 16 S rRNA gene sequencing [46]. A total of 1736,832 sequences from 30 samples corresponding to 304 (with ≥ 2 counts) unique ASVs were recovered. Data filtration and normalization (cumulative sum scaling) and the LEfSe technique were applied using the MicrobiomeAnalyst platform with default parameters [47]. Stacked bar charts showing

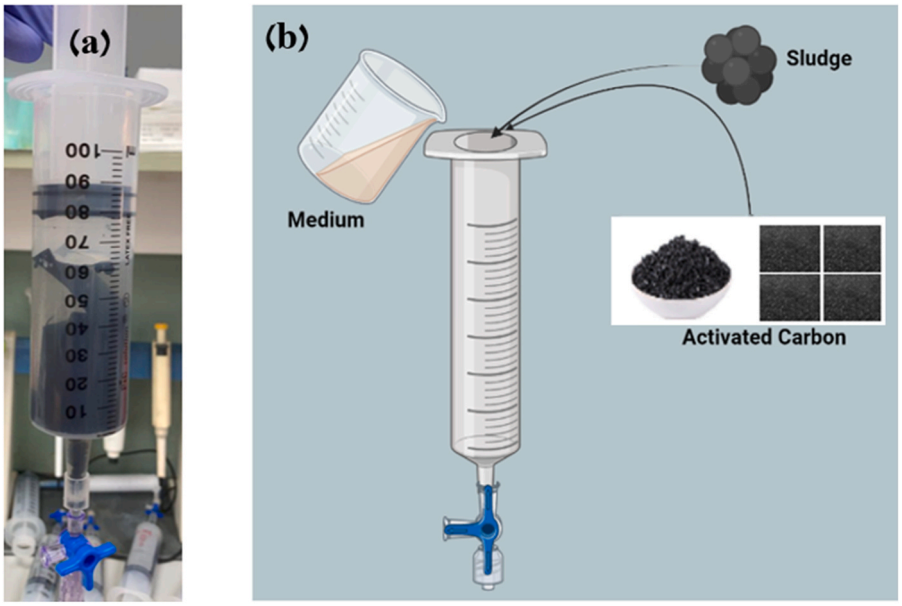


**Fig. 1.** (a) Biomethane potential (BMP) system and (b) Schematic illustration of the system used for measuring.

taxonomic classification of Bacteria and Archaea at the family level did not include taxa with relative abundance below 2%. Raw data from Illumina MiSeq sequencing are deposited to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject number PRJNA973252.

### 2.5. Analytical analysis

Methane ($CH_4$) content, $COD_{(s)}$ removal efficiencies, residual phenol concentration, pH, and EC were measured at the end of every BMP batch test. The methane content was analyzed using a gas chromatography GC system (Agilent 7890B Gas Chromatograph), combined with a thermal conductivity detector (TCD), equipped with Agilent DB-1ht GC Column, part number: 122–1111, 15 m length, 0.25 mm of inner diameter, 0.1 μm film thickness, where nitrogen was used as a carrier gas. The column temperature was fixed at 150 ºC, while the injector and detector were set at 200 ºC, and the volume of the injected biogas sample was 250 μl.

$COD_{(s)}$ was measured according to the 18th edition of *Standard Methods for the Examination of Water and Wastewater* [48]. The residual phenol concentration was evaluated with reverse-phase HPLC (Thermo Finnigan LLC Scientific Surveyor, California, USA) equipped with a PDA Plus detector (220–340 nm), according to [49]. A combo water tester (MRC Labs IP67, Israel) was used to measure the pH and EC, and a Zetasizer Ultra system (Malvern Panalytical Ltd. Instrumentation Company, Malvern, UK) was used to measure the zeta potential.

### 2.6. Statistical analysis

All statistical analyses were performed with the Prism 8.4.3 software. Data are presented as a mean ± standard deviation for the triplicate bioreactors in the BMP tests. A one-way Analysis of Variance (ANOVA) was used for the BMP test with different CC concentrations, while a two-way ANOVA was used for the BMP test with different AC types. For both BMP tests, a post hoc analysis with the Tukey's HSD range test was performed. Paired and unpaired t-tests were done for the zeta potential and sludge conductivity measurements, respectively. For all analyses, a P value $< 0.05$ was considered statistically significant.

## 3. Results and discussion

### 3.1. BioMethane potential (BMP) tests

#### 3.1.1. Optimization of carbon cloth concentrations

In this study, two BMP experiment sets were carried out to compare biomethane potential between different CCSW concentrations, and different types of AC. The accumulated biogas normalized to the initial COD, the biogas production rates with different concentrations of CCSW (1–20 g $L^{-1}$) and the control group (without CCSW) are shown in Fig. 2. The concentration range of 1.6–5.0 g $L^{-1}$ CCSW was associated with the highest biogas production rate. It is evident from these results that at dosage of CCSW below 1.6 g $L^{-1}$ (corresponding to 104 $cm^2$ $L^{-1}$), the adsorbent and conductive capability were insufficient to overcome phenol inhibition by adsorption and biodegradation processes, resulting in low accumulated biogas (Fig. 2a) and biogas production rates (Fig. 2b) compared to the carbon dosage of (1.6–5.0 g $L^{-1}$). Nevertheless, a slight decrease was observed with a dosage of 10 mg $L^{-1}$, and a significant decrease when an initial dosage of 20 mg $L^{-1}$ was applied. This suggests that, when adding high CCSW-dosage to the bioreactor, a strong adsorption capacity as well as intra-pore diffusion might significantly decrease the bioavailability of essential nutrients and biodegradable organic matter, and eventually to biological activity reduction (Fig. 2). High concentrations or dosages of CCSW was previously shown to negatively affect the DIET process [50–52]. It was speculated that the high amounts of AC adsorbed COD substances and reduced available substrates for methane conversion [53]. Other studies, which examined the addition of CC and GAC to AD reactors in order to increase the DIET process, applied mostly higher dosages than we use in our study (1.6 g $L^{-1}$) making our case more cost effective compared to the previous studies as shown in Table 2 [13,14,28].

#### 3.1.2. Effect of adsorption on phenol biodegradation and methane production

The optimal concentration of 1.6 g $L^{-1}$ ACs was used in these experiments to facilitate adsorption process and DIET mechanism. Adsorption tests were conducted for the different types of AC: GAC, CCK, and CCSW, with different phenol concentrations of 0–450 mg $L^{1}$.

Fig. 3 and Table 3 show that the adsorption capacity of CCSW was the highest at 207.5 mg/g, compared to 167 mg/g for GAC, and 157.6 mg/g for CCK, according to the Langmuir model (Table 3). These results are comparable to previous studies [58,59]. These different values can be the result of different physicochemical properties of the studied adsorbent (e.g. charge, available surface area) [60]. The

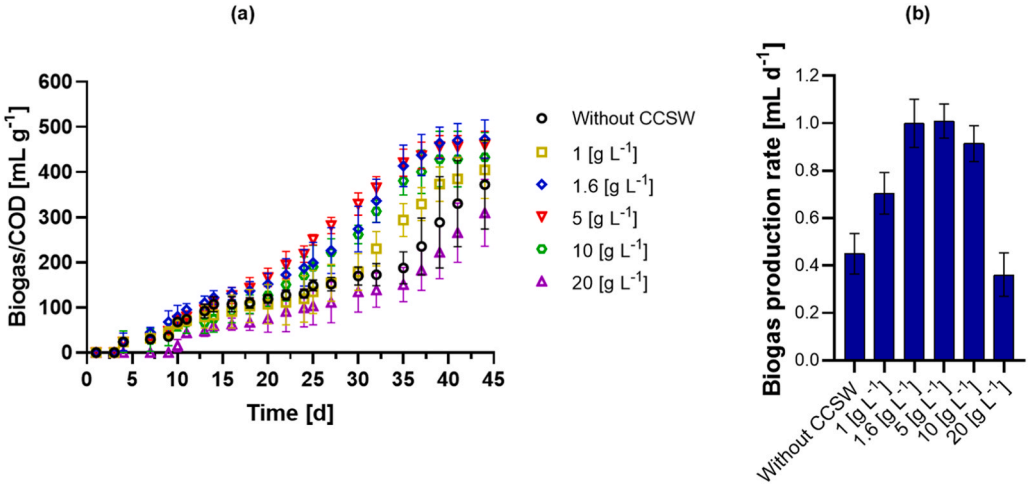


**Fig. 2. :** Accumulated Biogas (a) and biogas production rate (b) for different CCSW concentrations with medium containing 500 mg $L^{-1}$ phenol. Each point/column represents the mean of three bioreactors.

**Table 2**
CC and GAC dosage added to AD bioreactors and possible evidence supporting DIET occurrence.

| Type of activated carbon | Dosage | Effect on AD performance | AD mode | Reference |
|---|---|---|---|---|
| CC | 10 g $L^{-1}$ | - | Batch | [18] |
| CC | 977 $cm^2$ $L^{-1}$ | $CH_4$ production rate increased | Batch | [54] |
| CC | 83 $cm^2$ $L^{-1}$ | $CH_4$ production rate increased | Batch | [25] |
| CC | 5.2 g $L^{-1}$ | Higher resistance and recovery to acidic impacts | Semi-continuous | [23] |
| CC | 1465 $cm^2$ $L^{-1}$ | Increased methane production at high OLR | Semi-continuous | [55] |
| CC | 2.5 g $L^{-1}$ | $CH_4$ production rate increased | Continuous | [21] |
| CC | 833 $cm^2$ $L^{-1}$ | $CH_4$ production rate increased | Continuous | [15] |
| GAC | 25 g $L^{-1}$ | $CH_4$ production rate increased and significantly decrease in lag phase | Batch | [56] |
| GAC | 50 g $L^{-1}$ | $CH_4$ production rate increased | Batch | [54] |
| GAC | 40 g $L^{-1}$ | $CH_4$ production rate increased | Semi-continuous | [57] |
| GAC | 45 g $L^{-1}$ | $CH_4$ production was enhanced, better performance and higher stability in wide temperature range | Continuous | [27] |
| CC | 1.6 g $L^{-1}$ (104 $cm^2$ $L^{-1}$) | $CH_4$ production rate increased | Semi-continuous | This study |

addition of salts and nutrients led to a maximal adsorption capacity 171 mg/g for CCSW (Fig. S5), which is slightly lower than the capacity of phenol in DD water (207.5 mg/g). We assume that a similar reduction in the adsorption capacity will also occur for the different types of AC.

From Fig. S1 (supplementary material), it appears that CCSW has also the fastest adsorption kinetics at high and low phenol concentrations (500 and 100 mg $L^{-1}$, respectively).

Fig. 4 shows the accumulated biogas in the three runs of batch experiments of the different types of AC, including two control sample-types: i) medium without AC (referred to as Medium), and ii) medium without phenol and AC, containing only peptone and yeast as an organic source (referred to as Medium-No phenol).

In the first run, all samples (except the medium without phenol) showed a lag time of four days in producing biogas, demonstrating the initial inhibition of biological activity due to moderate phenol concentrations of 500 mg $L^{-1}$ [3,61]. Nevertheless, eventually all AC-containing bioreactors produced significantly more biogas compared to the control starting from day 15, leading to high differences in the accumulated biogas at the end of the first run relative to both controls. Specifically, 54.33, 50.33 and 44.66 mL were produced in the bioreactors containing CCK, CCSW and GAC respectively, compared to

**Table 3**
Parameters of phenol adsorption isotherms.

| | Langmuir Model $q_e = \frac{q_m b C_e}{1 + b C_e}$ | Freundlich Model $q_e = \frac{q_m b C_e}{1 + b C_e}$ | Incubation time [h] |
|---|---|---|---|
| GAC | $q_m$=167.0 | K=38.34 | 36 |
| | b=0.1305 | *1/n*=0.2964 | |
| | $R^2$=0.9405 | $R^2$=0.9845 | |
| | AIC=138.3 | AIC=106.1 | |
| CCK | $q_m$=157.6 | K=32.27 | 18 |
| | b=0.0664 | *1/n* =0.2965 | |
| | $R^2$=0.9391 | $R^2$=0.9428 | |
| | AIC=132.9 | AIC=131.4 | |
| CCSW | $q_m$=207.5 | K=38.31 | 18 |
| | b=0.0698 | *1/n* =0.3332 | |
| | $R^2$=0.9956 | $R^2$=0.9724 | |
| | AIC=81.89 | AIC=125.8 | |
| | b=0.0664 | *1/n* =0.2965 | |
| | $R^2$=0.9391 | $R^2$=0.9428 | |
| | AIC=132.9 | AIC=131.4 | |

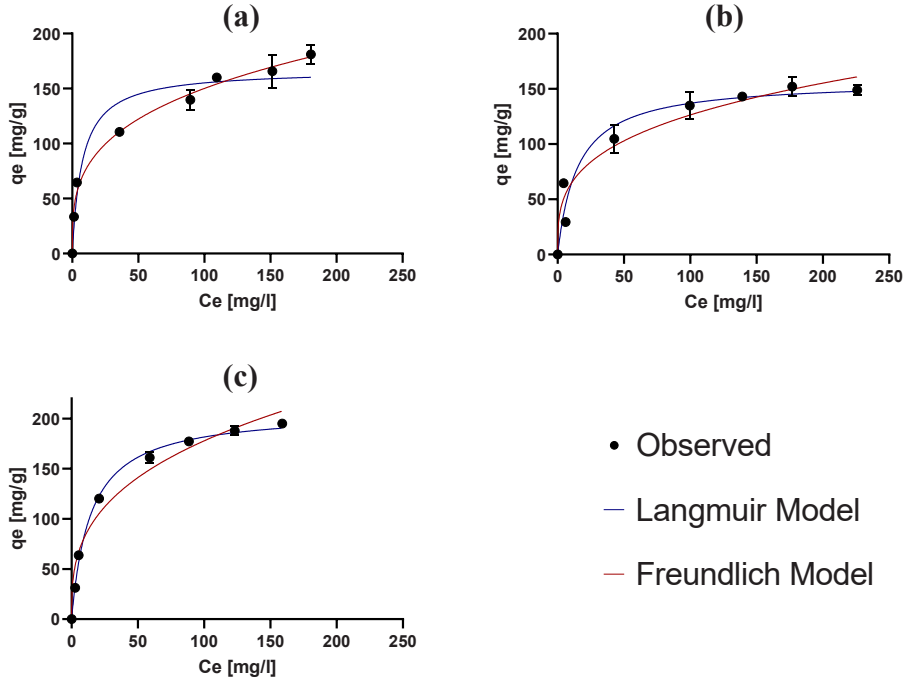


**Fig. 3.** Adsorption isotherms of phenol on different AC: GAC (a), CCK (b), and CCSW (c).

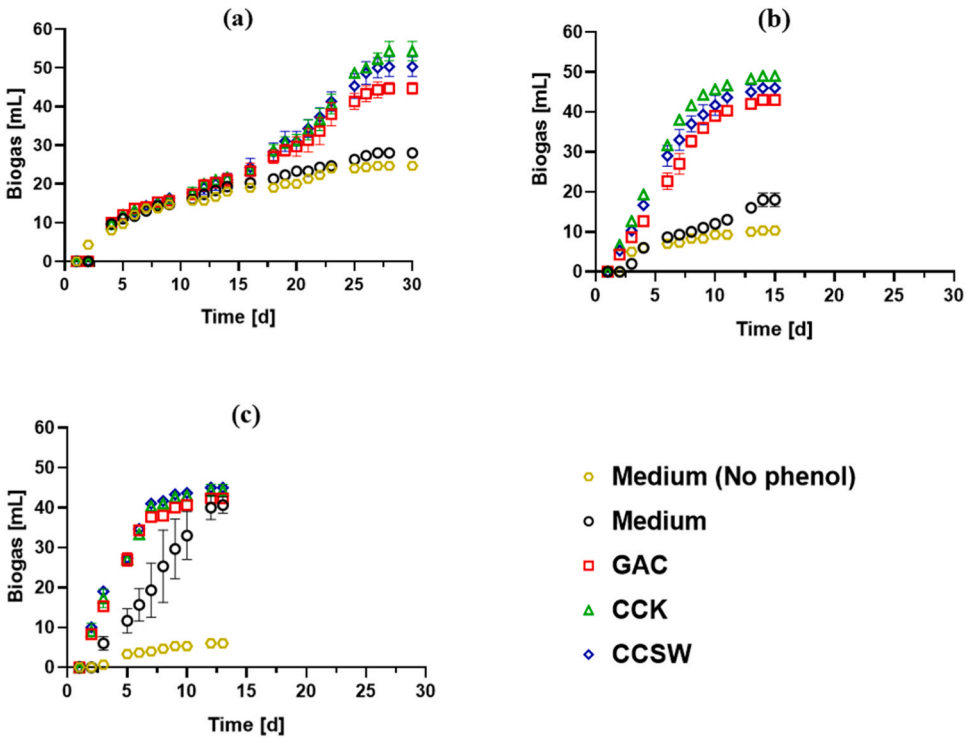


**Fig. 4. :** Accumulated biogas for all different AC types at 500 mgL$^{-1}$ phenol in the first (a), second (b), and third (c) runs. Each point represents the mean of three bioreactors.

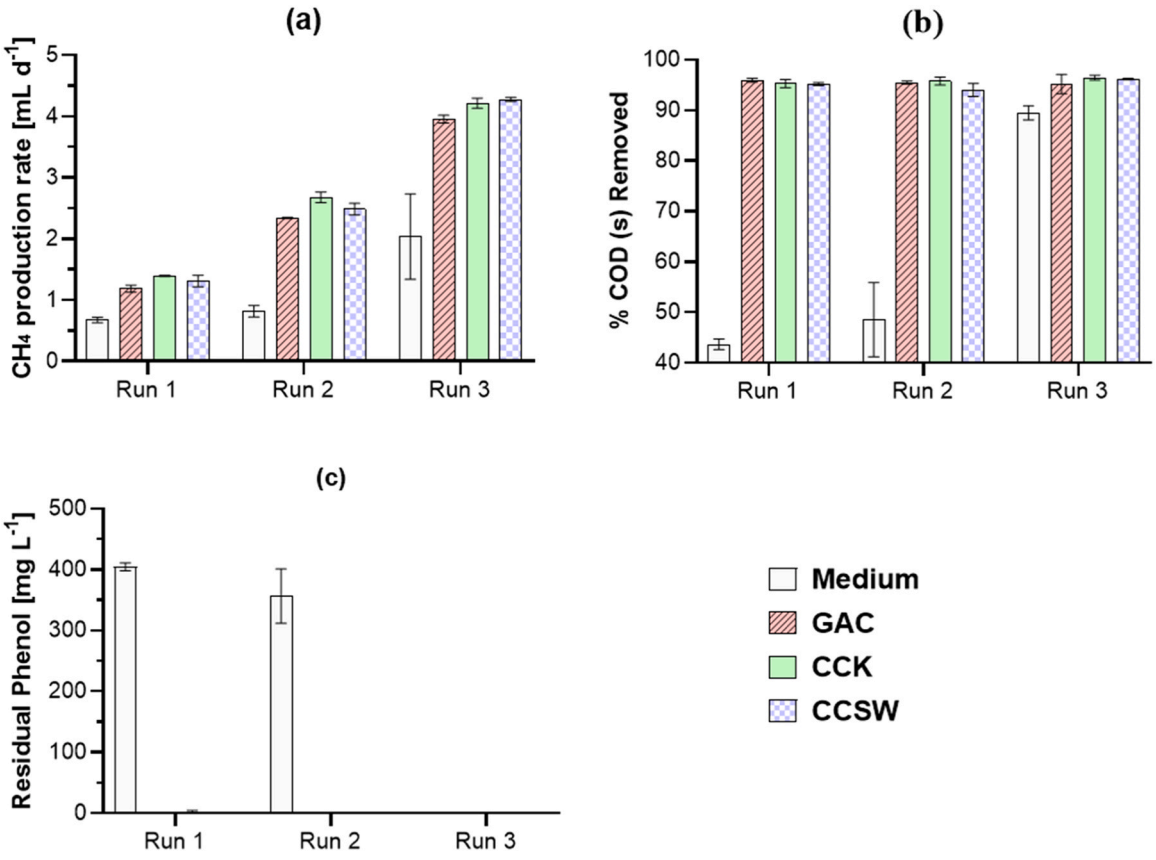


**Fig. 5. :** $CH_4$ production rate (a), % $COD_{(s)}$ removed (b), and residual phenol (c) for all different AC types at 500 mg L$^{-1}$ phenol. Each column represents the mean of three bioreactors.

28 mL produced in the control reactor (Medium). Moreover, the controls in the first run produced similar biogas volume, suggesting that phenol biodegradation was not achieved in the Medium control (Fig. 4a). Also, in the second run, biogas accumulation substantially differed between the controls and the AC-containing reactors (Fig. 4b). However, by the third run, the Medium-control reactor appeared to gradually adapt to phenol (Fig. 4c). These findings are in good agreement with the results of Wang et al., [22] indicating that adsorption capacities were the main determining parameters of biochar, eventually reflected in the differences in organic conversion and biogas yield. Another important interpretation could be that the poor conductivity and electron transfer capacity of biochar limits its role as an electron conduit for DIET. Moreover, the mediated interspecies electron transfer via hydrogen and formate was still considered as the primary mechanism for electron transfer. In our case the mediated interspecies electron transfer might be the dominant mechanism for the less conductive CCK.

At the end of each run, the methane content was measured and the $CH_4$ production rates were calculated and presented (Fig. 5a). It was found that the AC-added bioreactors had $CH_4$ production rates in the ranges of 1.18–1.39, 2.33–2.67, 3.95–4.27 mL $d^{-1}$ in the first, second, and third runs, respectively, compared to 0.67, 0.81, 2.03 mL $d^{-1}$ in the control group (Fig. 5a); resulting in an increment of about twofold the $CH_4$ production rates compared to the control. Improved methane yield and organic matter removal efficiency were also previously reported for laboratory-scale AD reactors with different carbon-based conductive materials [55]. Additionally, in the control samples, the removed $COD_{(s)}$ was 43.68%, 48.65% (Fig. 5b), and the residual phenol concentrations were 404 and 356 mg $L^{-1}$, in the first and second runs, respectively (Fig. 5c). While in the AC-added bioreactors, removed $COD_{(s)}$ remained above 95% (Fig. 5b), with negligible phenol concentrations throughout the entire period of the experiment (Fig. 5c). This implies that adsorption decreased phenol inhibition and contributed to significant and fast phenol reduction through biodegradation and $CH_4$ production processes. Only in the third run of the control sample, biodegradation of phenol was observed, leading to a $COD_{(s)}$ removal of 89.52% with negligible phenol concentration remaining (Fig. 5b-c). These results indicate that incorporating AC into anaerobic systems reduces phenol biological inhibition and enhances the AD process (Fig. 5a-c), supported by DIET-mediating microorganisms (as discussed below).

Our results suggest synergetic mechanisms of the two processes: adsorption of phenol on the AC and enhanced anaerobic biological activity of the adsorbed phenol through DIET. These mechanisms were previously suggested and observed by Li et al. (2021), who used biochar and GAC to intensify anaerobic phenol degradation) [1]. It should be noted that no significant differences were found between the various AC types in our study. This latest observation converses the outcomes of Barua et al. (2017), where methane production rate of the AD systems with bio-based carbon cloth was lower than that of the AD systems with biochar and GAC additives [62]. A possible explanation might be that the physicochemical properties of these AC types are relatively similar.

It can be also noted that the rate of $CH_4$ production gradually increased after each run (Fig. 5a). This is apparently due to biofilm growth on the AC surface. The growth of microorganisms on the surface of AC was evident using environmental scanning electron microscopy (ESEM- TESCAN MIRA3), where direct contact of microorganisms on the CCSW surface and within the pores were observed (Fig. S4, Supplementary material). These images also support the axiom that microbial biofouling attached to CCSW increased the zeta potential (from −13 mV to −21 mV) (Fig. S3b, supplementary material). The change in conductivity of the sludge was also measured (Fig. S3a, Supplementary material), showing CCSW-added bioreactors were almost two-fold higher in conductivity than that of the inoculum (464 mS/cm vs. 261 mS/cm, respectively). These changes are possible when CCSW promotes microbial processes that participate in DIET to transfer free electrons between microbial cells or conductive surfaces, similar to the results of Lei et al. [15].

### 3.2. Microbial community analysis

The relative abundance of archaeal community at the family taxonomic level of all treatments (attached on the CCSW, SS-CCSW, SS-B, and SS-Time 0), throughout the experiment, showed that *Methanosaetaceae* was the most abundant methanogen, particularly in the Attached treatment compared to the SS treatments (54%, 62%, 61% vs. 47%, 44%, 44%, in the first, second, and third runs, respectively, and 47% in SS-Time 0, Fig. 6a-c). Furthermore, the relative abundance of *Methanosaetaceae* increased from the first run through to the third run only in the Attached treatment, while the SS treatments remained unchanged. *Methanosaetaceae* is one of two methanogenic families that can utilize acetate as their carbon source for methanogenesis [63]. It has been found that species within this family (namely, *Methanothrix spp.*) play a major role in global methane emissions, through $CO_2$ reduction to methane, by creating syntrophic interactions with different exoelectrogenic bacterial species, such as *Geobacteraceae,* during the DIET process [34,64–66]. Conductive materials are frequently added to anaerobic bioreactors dominated by *Methanosaetaceae* to enhance DIET between exoelectrogens and methanogens [18,56,67,68]. Ultimately, the methanogens receive free electrons via c-type cytochromes on a conductive surface [28]. In a study similar to ours, Zhang et al., 2017 [69] reported that the addition of GAC into a bioreactor stimulated *Methanosaetaceae* abundance. Surprisingly, it was found that the growth of *Methanothrix thermoacetophila* alone or in co-culture with *Geobacter metallireducens* was severely impaired by the presence of GAC compared with magnetite additions [70]. However, a different study showed enhanced DIET between *Geobacter metallireducens* and *Methanosarcina barkeri* after adding GAC during anaerobic digestion [56]. Indeed, the latter study found that GAC provided higher conductivity between cells than bio-electrical connections (conductive nanowires), which could be species specific. This emphasizes the significant contribution of applying conductive materials in anaerobic digestion systems for interspecies electron transfer and DIET stimulation [71]. Another family, *Methanoregulaceae*, is a known syntrophic methanogenic family that is responsible for the degradation of VFAs while consuming hydrogen [72]. In addition, Kim [72] showed that the addition of magnetite significantly contributed to the enrichment of *Methanoregulaceae*, which was more abundant in the Attached treatment than in the SS treatments (6.3%, 4.9%, 5.5% vs 3.2%, 3.6–3.1%, respectively, Fig. 6). *Woesearchaeales* was mostly found in the Attached treatment in the first run (2.4%). This group is known as a syntrophic methanogen, and its abundance was correlated with *Methanoregulaceae* in anaerobic sludge [73]. Overall, it can be seen that the addition of CCSW to the anaerobic bioreactor increased the presence of syntrophic-associated families that facilitate DIET (*Methanosaetaceae* and *Methanoregulaceae*) in the Attached treatment, instead of other methanogenic members that are commonly detected in anaerobic sludge such as *Methanobacteriaceae* and *Methanomassiliicoccaceae* [74,75].

Fig. 6d-f show that the composition of the bacterial community in the Attached treatment is significantly different from that in the SS treatments. Furthermore, while the bacterial community in the Attached treatment continuously developed during the batch sequencing, that of the SS treatments remained almost unchanged throughout the experiment. *Syntrophorhabdaceae* was the most abundant family in the Attached treatment during the entire experiment (20%, 19%, 19% in the first, second and third runs, respectively), while in the SS treatments, abundance was significantly less (< 1.12–5.2% for all runs). *Syntrophorhabdaceae* is a family largely represented by abundant bacteria within anaerobic ecosystems that decompose aromatic compounds such as phenol [76]. Members of this family are known for their syntrophic growth with methanogenic archaea, providing these archaea with hydrogen and formate, which are essential for methane production [77]. *Desulfuromonadaceae* are also known for their ability to break down aromatic compounds [78]. Members of this family were shown to extracellularly transfer electrons to associated syntrophic species. In this

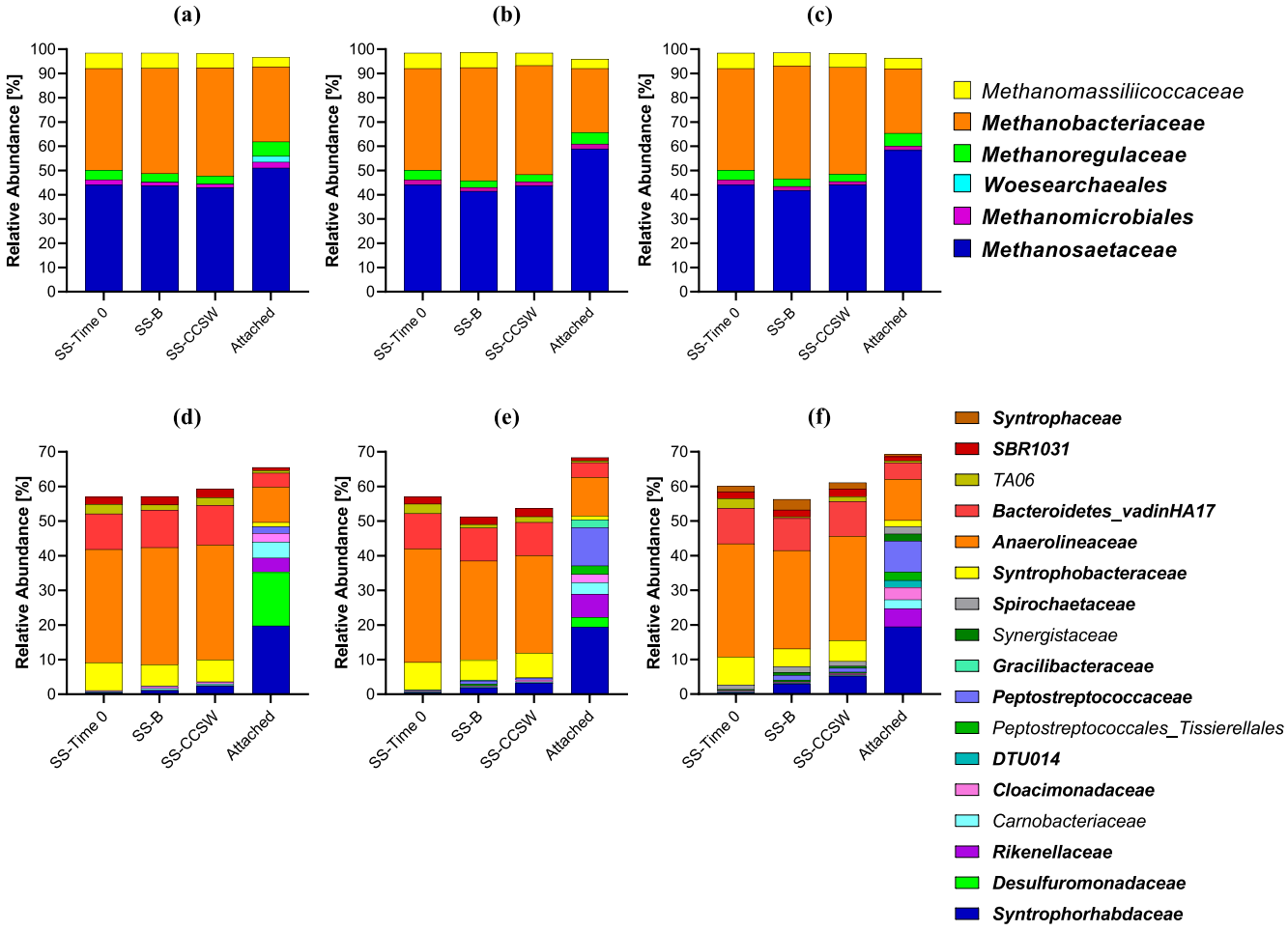


**Fig. 6. :** Family-level relative abundance of archaea in the first (a), second (b) and third (c) runs and bacteria in the first (d), second (e), and third (f) runs. Relative abundance below 2% are not shown. Syntrophic families are bolded in the legend. Each family represents the mean of triplicates.

study, *Desulfuromonadaceae* was also highly abundant (15.3%) in the first run of the Attached treatment, but negligible in the SS treatments (< 1% for all runs). *Rikenellaceae*, which was found in high abundance in the Attached treatment (4.2%, 6.8%, 5.2%), but barely present in the SS treatments, is known as a family that participates in the DIET process with methanogenic families, and often observed to colonize conductive surfaces [79,80]. Furthermore, while the relative abundance of *Peptostreptococcaceae* increased from the first run compared to other runs in the Attached treatment (3.2%, 11%, 9% respectively), it remained negligible in the SS treatments for all runs. *Peptostreptococcaceae* is known to have the ability to attach to other bacteria such as *Clostridiaceae*, and to produce aggregates that contribute to the adhesion of other microorganisms to AC. Moreover, *Peptostreptococcaceae* can receive free electrons to reduce carbon dioxide for the production of acetate, which contributes to the methanogenesis pathway [81]. Additional families of bacteria were observed throughout the experimental period in the Attached treatment that were not observed in the SS treatments, such as *DTU014*, *Cloacimonadaceae*, and *Gracilibacteraceae*. *Gracilibacteraceae* and *DTU014*, which are known to participate in the DIET process on conductive surfaces. Alternatively, *Cloacimonadaceae* is known as a syntrophic family that produces hydrogen for methanogenic archaea [58,80,82]. While the bacterial community composition in the Attached treatment was diverse, and composed of syntrophic families that facilitated DIET and degraded phenol, the bacterial community in the SS treatments was mainly composed of three families commonly found in anaerobic environments and perform the anaerobic degradation of complex hydrocarbons (*Anaerolineaceae*, *Syntrophobacteraceae*, and *Bacteroidetes-Vadinha*) [16]. The DIET process was initially confirmed using various molecular analyses [34], which provide direct evidence to the presence of DIET-related microbes. However, other approaches could be applied for this purpose, including methane production, organics conversion, and shortened start-up time [71]. In this study, we support the occurrence of the DIET process through the combination of microbial community analysis and methane production measurements. Overall, the composition of the bacterial population in the Attached treatment was highly dynamic, while that in the SS treatments did not exhibit significant changes, and remained similar to the inoculum (SS-Time 0 treatment). Moreover, the distribution of the Attached community was mainly composed of DIET-related microbes, which were less apparent in the suspended communities.

## 4. Conclusions

This study shows the application of AC to stimulate the DIET process in anaerobic bioreactors, leading to an increase in the phenol biodegradation at high concentrations. The optimal concentration of carbon cloth type CCSW was found to be 1.6 g $L^{-1}$. This concentration supported DIET and adsorption processes, and at the same time, is expected to minimize costs and the ecological footprint of the treatment. The addition of all types of AC to the bioreactors contributes to a significant acceleration of the methane production rate, which co-occurred with high $COD_{(s)}$ removal and full anaerobic biodegradation of phenol. According to the adsorption isotherms and kinetics reported in the supplementary material, the enhancement of phenol biodegradation is due

to synergetic adsorption-biodegradation processes, where a significant and fast reduction of phenol via adsorption contributes to decreasing the inhibition effect, followed by biological AD of the adsorbed phenol by stimulating DIET process. This observation was further corroborated by a microbial community analysis that revealed a wide variety of microorganisms known to facilitate the DIET process and support phenol biodegradation. These microorganisms were found to be abundant on conductive surfaces such as AC and magnetite. This biofouling significantly increased the zeta potential of the CCSW (supplementary material), which provides an electrically conductive surface for the transfer of free electrons between syntrophic microorganisms. Furthermore, the addition of CCSW to the bioreactor significantly increased the conductivity of the sludge. This study demonstrates for the first time the use of CC for the stimulation of DIET to enhance phenol biodegradation. These results provide scientific outcomes for the future application of CC in biological systems, such as anaerobic membrane bioreactor (AnMBR) and UASB for the treatment of inhibitory compounds.

## CRediT authorship contribution statement

**Shimshoni Stav:** Writing – original draft, Visualization, Validation, Investigation, Data curation. **Yanuka-Golub Keren:** Writing – review & editing, Validation, Methodology, Formal analysis. **Baransi-Karkaby Katie:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Azaizeh Hassan:** Writing – review & editing, Project administration, Formal analysis. **Sabbah Isam:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Hassanin Mahdi:** Validation, Methodology, Data curation.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Isam Sabbah reports financial support was provided by European Union.

## Data Availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2024.112222.

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